

# التعرف الجزيئي على بعض مسببات مرض القوباء الحلقية المعزولة من الابقار في مدينة الديوانية

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الملف	SES-OF-RING-WORM-ISOLATED-FROM-CATTLE-IN-AL-DIWANIYA-REGION.DOC (889.89K)		
الوقت المرسل	03-أبريل-2017 12:03 م	عدد الكلمات	3884
معرف الارسال	793493138	عدد الرموز	22547

## التعرف الجزيئي على بعض مسببات مرض القوباء الحلقية المعزولة من الأبقار في مدينة الديوانية

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الكلمات الافتتاحية : القوباء الحلقية , انواع الترايكوفاييتون , تفاعل السلسلة المتعدد , الشجرة الوراثية .

الخلاصة : مرض القوباء الحلقية مرض فطري يسبب خسائر اقتصادية كبيرة خصوصا في الحقول التي تستخدم لإنتاج الجلود الطبيعية نتيجة انخفاض في إنتاج الجلود السليمة , ونقصان في إنتاج الحليب واللحم , هذا المرض يتسبب بواسطة عدة اجناس من الفطريات هي الميكروسبوريوم والابديرموفاييتون والترايكوفاييتون. الترايكوفاييتون واحدة من الفطريات الاكثر شيوعا والمحبة للحيوانات والتي تستعمر على الانسجة المتقرنة للانسان والحيوان. نتائج دراستنا اظهرت بان نسبة العزلات الكلية الفطرية المعزولة من الأبقار كانت 34.2% بواسطة استخدام الزراعة على الاوساط الزراعية, بينما كانت نسبتها 100% بواسطة استخدام الطرق الجزيئية ( تفاعل السلسلة المتعدد). في هذه الدراسة تم عزل نوعين من الفطريات هم الترايكوفاييتون فيريوكوسم وفرطيات الترايكوفاييتون ربرم. كانت نسبة الترايكوفاييتون ربرم 33.3% بينما كانت نسبة الترايكوفاييتون فيريوكوسم كانت 66.6% تحليل الشجرة الوراثية لانواع الترايكوفاييتون بواسطة استخدام بادئ صمم لهذا الغرض لجين ( ار ان اي) لتشخيص فطريات الترايكوفاييتون فيريوكوسوم والترايكوفاييتون ربرم .

على كل , فان فطريات التترايكوفاييتون ربرم اظهرت تشابه كبير مع الراكوفاييتون راوبيتشكي بنسبة 99% والترايكوفاييتون كاني بنسبة 99% وترايكوفاييتون مكنيني بنسبة 98% .

بينما الترايكوفاييتون فيريوكوسوم اظهرت تشابه كبير مع الترايكوفاييتون مكنيني بنسبة 93% والترايكوفاييتون سيركونفيليتوم بنسبة 92% واخيرا تسابه مع الترايكوفاييتون انترديجتال بنسبة 93% .

نسبة التشابه ما بين العزلات المعزولة في هذه الدراسة مع تحليل الشجرة الوراثية كانت بنسبة 100% بواسطة استخدام مورثة ( اران اي) .

## **Molecular identification of some causes of ringworm isolated from cattle in Al-Diwaniya region**

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**Keyword: Ring worm, Trichophyton spp, PCR, phylogenic.**

### **Abstract :**

The ringworm is fungal disease results in high economic losses particularly in the fields that used for leather production due to the down grading of healthy skin; a decrease in meat and milk production. this disease caused by several fungi genera like microsporium, epidermophytone, and trichophyton.

*Trichophyton spp* is one of fungi most common zoophilic and dermatophyte fungi that parasitizes and keratinized tissues in human and animal.

Our study results showed that percentage of total isolated fungi from infected cattle are (34.2%) by using classical media culture, while it was (100%) using molecular methods (PCR ). in this study isolated two type of fungi are *Trichophyton verrucosum* and *Trichophyton rubrum*.

Percentage of *Trichophyton rubrum* that diagnosed in cattle in our study are (33.3%); while was (66.6%) in *Trichophyton verrucosum*.

phylogenetic analysis tree of *Trichophyton spp* was done by using a primer designed for specific rRNA gene for detection of *Trichophyton rubrum* and *Trichophyton verrucosum*.

whenever *Trichophyton rubrum* showed high likely similarity with *Trichophyton raubitschekii* (99%), *Trichophyton kanei* (99%) and *Trichophyton megninii* (98%).

while *Trichophyton verrucosum* show similarity with *Trichophyton megninii* (93%), *Trichophyton circonvolutum* (92%) and finally similarity with *Trichophyton interdigital* (93%).

Percentage of similarity of the isolates of this study with phylogenic tree analysis is (100%) by using rRNA gene.

### **Introduction:**

Dermatophytes cause surface infections of the skin, characterized by the non-inflammatory lesions due to *Trichophyton spp* as the most common (1).

Superficial infection in human and animals caused by dermatophytes are spread all the animals and all the age . The diseases have a zoonotic importance because the infection transmitted between the animals and human by contact (2).

In dairy beef production and also in public health, dermatophytosis may be of economic importance due to costs of treatment, decreased skin value and weight (3-4) and its incremented for high economic losses in cattle due to damages and loss of the good leather (5).

*Trichophyton verrucosum* is a zoophilic fungus and is a cause factor of disease of the ringworm in human and domestic animals e.g a camel and cow(6). Direct contact is common methods to spread the disease; also *T. verrucosum* also doing financial loss in field animals (7). It is transmitted to human through direct contact with contaminated cattle or its products to result inflammatory lesion in the head, face etc(8).

*Trichophyton rubrum*, is the spreadable and common factor of mycosis over the worldwide, basically infect all the creature(9). This fungus commonly causing cutaneous diseases and considered the discovery of its genomic structure may reduce the health costs of those who affected with different forms of fungal disease caused by *T. rubrum* (10).

*T. rubrum* causes cutaneous infection and deformity of the shape (10). Our informations are very little about the mechanism or pathogenesis of this fungus (11). It is usually not life-threat and don't cause acute infection, but usually are chronic infections, reinfection, but it's difficult to heal. The fungal pathogen's can formed and secrete specific enzymes

considered as pathogenic and virulence factor (12). *T. rubrum* transmitted to the animals and the human skin via contact by keratin layers (10)(12).

*Trichophyton verrucosum* is the animals' fungi causing in cow dermatophytosis in humidity environments of our plant and the goat and sheep but less percentage (13,14).

The Ringworm disease occurs in calves more prevalent than adult age(cow) and is speedily spreading between the animals of the one herd by infected flockes, like hyphae, and fungal spores called arthrospores. The disease occurs in all worldwide (15,16).

Furthermore, this disease in cattle also has been reported and confirmed in ruminants as examples goat and sheep (13,14).

PCR technique is one of the unique and very sensitive ways which used for detection the dermatophytosis disease, such as tinea unguium disease and tinea pedis disease (17,18).

(19) uses real-time PCR to identification of the organisms a are assay with universal primer kit and its probe for whole the fungi, also the specific primer used to detect *T. rubrum* as accuracy (19).

Our study has been designed to diagnose and confirm the molecular Identification types of *Trichophyton* spp which collected from the skin of cow by some media culture like SDA agar and PCR then tested by phylogeny analysis tree to confirm the final diagnosis these fungi.

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## **Materials and Methods:**

### **Sample collection and culture:**

The study was conducted by collection samples from cattle from different regions of Al-Diwaniya, the sample collected was done by using scraping by sterile scalpel, keep the samples in a petri dish, sent to the lab.

Samples were treated with several drops from solution KOH (10%) were added to each sample to dissolve the keratin for (2) hours for complete dissolving and separating the colony. tocoopherol cotton blue was used to staining the fungi and examined under the microscope at 40X, then grew on SDA media where kept at 25°C - 10 days (20)(21).

### **Bacterial genomic DNA extraction:**

Genomic pellet DNA was extracted from fungal samples by using (specific kit called EZ-10 Spin Column).

Fungal cell pellets were collected from the 1ml fungal culture by centrifugation then ground in liquid nitrogen using a pestle and transferred to a clean 1.5 ml small centrifuge tubes. Extraction of fungal DNA was done according to company instructions. After that, the extracted fungal genomic was tested by Nanodrop spectrophotometer apparatus, after store in -30C at freezing for PCR assay time.

### **Polymerase chain reaction methods:**

PCR technique has done for diagnosis dermatophytes fungi which called *Trichophyton* spp. depended on small subunit ribosomal RNA gene by using specific primers that designed in this study by using NCBI-GenBank and primer3 plus site design online. These primers were prepared and made by (Bioneer com. Korea) as following table(1).

**Table(1): The primer used in this study with its sequence, and it's pb ( amplicon ).**

<b>Primer</b>	<b>Sequence</b>	<b>Amplicon</b>	<b>GenBank</b>
<b>small subunit ribosomal RNA gene</b>	<b>GATCAGCGTTCCATC AGGGG</b>	<b>540bp</b>	<b>AF168126. 1</b>
	<b>GAGAGATTTGGGGGA AGGCC</b>		

The PCR solution mix was prepared by the company called (Bioneer). The PCR solution kept at small tube containing freeze-dried pellet of DNA and the PCR master mixture reaction was done according to kit instructions, then filled to the top by the PCR premix tube by deionized water into 20µl and used vortex centrifuge (Bioneer. Korea) for good mixing. The reaction was done in a thermocycler system (MygeneBioneer) by orders we can sit it on the control panel the

following thermocycler conditions; The products were examined by electrophoresis on a 1% agarose gel under UV light.

**DNA sequencing method:**

genetic sequencing of subunit ribosomal RNA gene by using analysis of phylogenetic relationship and study level of alignment by mega multiple sequence software alignment programs. At 540bp product was purified from the gel by using (Gel Extraction Kit). The purified rRNA gene PCR product was sent to DNA sequence Company in Korea for doing the DNA sequencing using 18-rRNA primer designing by (ABDNA sequencing system).

**Results :**

In this study used classical methods and molecular methods to diagnosis of fungi , used SDA agar for culture that keep at 25°C for (10) days as figure (1), however the tested (35) isolates by culture methods produce the positive is (12)(34.2%) while these(12) isolates used molecular detection methods for confirm these (12)(100%) isolates see the table (2) .



**Figure (1): Dermatophytes fungal growth cultures on (SDA) that incubated at agar. Where A: Dermatophyte *Trichophyton rubrum* and B: *Trichophyton verrucosum* according to PCR technique and phylogenetic tree analysis results.**

**Table (2): Prevalence of dermatophytes fungi that detected by two methods shows its number and percentage.**

<b>Method</b>	<b>No .of tested isolates</b>	<b>Positive</b>	<b>Prevalence (%)</b>
<b>Culture method</b>	<b>35</b>	<b>12</b>	<b>34.2%</b>
<b>Dermatophytes fungi based PCR</b>	<b>12</b>	<b>12</b>	<b>100%</b>

*Trichophyton rubrum* and *Trichophyton verrucosum* were (2) isolate out of (35) isolate By use PCR methods.

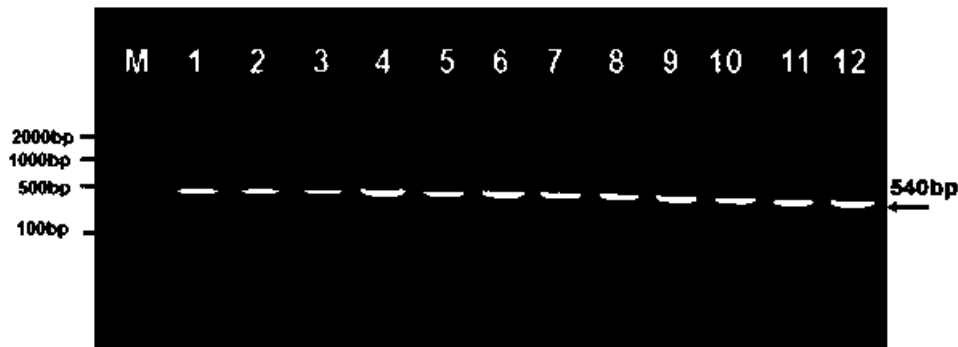
PCR used to determination species of fungi, *trichophyton rubrum* is (4) out from (12)(33.3%) tested isolates, while *Trichophyton verrucosum* is (8) out from (12)(66.6%) tested isolates the table (3). it has been read on electrophoresis also Figure (2).

**Table(3): The prevalence of dermatophytes fungi species that detected by phylogenetic tree analysis:**

<b>Fungal isolates species</b>	<b>No .of tested isolates</b>	<b>Positive</b>	<b>Prevalence (%)</b>
<i>Trichophyton rubrum</i>	<b>12</b>	<b>4</b>	<b>33.3%</b>
<i>Trichophyton verrucosum</i>	<b>12</b>	<b>8</b>	<b>66.6%</b>

After making DNA extraction, PCR, and finally used electrophoresis to read the results of agarose gel electrophoresis see figure(2).





**Figure (2):** this picture show under UV light by use Agarose gel in electrophoresis analysis apparatus that shows the PCR product analysis of small subunit ribosomal RNA gene in *Trichophyton* spp. isolates. M: marker ( range between 100 to 2000bp), lane (1-12) some of positive *Trichophyton* spp. in (540bp) PCR product size.

phylogenic of *trichophyton verrucosum* has been making Homology sequence identity by use rRNA gene according to NCBI-BLAST site show table (4) highly sensitivity to accuracy molecular detection.

**Table (4): NCBI-Blast Homology sequence identity for rRNA gene in *Trichophyton* sp. isolate-1 with NCBI-BLAST *Trichophyton* spp.:**

Sequences producing significant alignments:

Select: All None Selected:0

Alignments

	Max score	Total score	Query cover	E value	Ident	Accession
1. <a href="#">Trichophyton verrucosum genomic rRNA gene (LN614528.1)</a>	975	1603	100%	0.0	100%	Query_183680
2. <a href="#">Trichophyton megnini genomic rRNA gene (AF170464.1)</a>	769	1683	96%	0.0	93%	Query_183686
3. <a href="#">Trichophyton circinvolutum genomic rRNA gene (AJ270791.1)</a>	758	1520	96%	0.0	92%	Query_183684
4. <a href="#">Trichophyton interdigitale genomic rRNA gene (AJ270790.1)</a>	751	1501	98%	0.0	93%	Query_183683
5. <a href="#">Trichophyton violaceum genomic rRNA gene (AJ270796.1)</a>	749	1471	96%	0.0	92%	Query_183682
6. <a href="#">Trichophyton rubrum genomic rRNA gene (AJ270806.1)</a>	749	1528	96%	0.0	92%	Query_183681
7. <a href="#">Trichophyton raubitschekii genomic rRNA gene (AF170468.1)</a>	747	1635	96%	0.0	92%	Query_183687
8. <a href="#">Trichophyton kane genomic rRNA gene (AF170460.1)</a>	747	1593	96%	0.0	92%	Query_183685

Trichophyton verrucosum genomic rRNA gene (LN614528.1)  
 Sequence ID: Query\_133148 Length: 625 Number of Matches: 42

Range 1: 76 to 615		Graps			Next Match
Score	Expect	Identities	Gaps	Strand	
975 bits(1080)	0.0	540/540(100%)	0/540(0%)	Plus/Plus	
Query 1	GATCAGCGTTCCATCAGGSGTGTGCAGATGTGCGCCGGCCTTACGCCCCATTCTTGTCTA				60
Sbjct 76	GATCAGCGTTCCATCAGGSGTGTGCAGATGTGCGCCGGCCTTACGCCCCATTCTTGTCTA				135
Query 61	CCTTACTCGGTGCTTGGCTCGCGCGCCGGCTCTCCCCGGAGAGTGGTCCGGCGAGCCTCT				120
Sbjct 136	CCTTACTCGGTGCTTGGCTCGCGCGCCGGCTCTCCCCGGAGAGTGGTCCGGCGAGCCTCT				195
Query 121	CGGGGGCTTTAGCTGGATCGCCCGCCGGAGGACAGACATCAAAAAATCTTGAAGAGCT				180
Sbjct 196	CGGGGGCTTTAGCTGGATCGCCCGCCGGAGGACAGACATCAAAAAATCTTGAAGAGCT				255
Query 181	GTCAGTCTGAGCGTTAGCAAGCAAAATCAGTTAAAATTTCAACAACGGATCTCTTGGTT				240
Sbjct 256	GTCAGTCTGAGCGTTAGCAAGCAAAATCAGTTAAAATTTCAACAACGGATCTCTTGGTT				315
Query 241	CCGGCATCCATGAAGAACGCACCGAAATGCGATAAGTAATGTGAATTGCAGAAATCCGTG				300
Sbjct 316	CCGGCATCCATGAAGAACGCACCGAAATGCGATAAGTAATGTGAATTGCAGAAATCCGTG				375
Query 301	AATCATCGAATCTTTGAACGCACATGCGCCCTCTGGTATTCGGGGGGCATGCTGTTC				360
Sbjct 376	AATCATCGAATCTTTGAACGCACATGCGCCCTCTGGTATTCGGGGGGCATGCTGTTC				435
Query 361	GAGCGTCAATTTCAACCCCTCAAGCTCGGCTTGTGTGATGGACGACCGTCCGGCCCCCT				420
Sbjct 436	GAGCGTCAATTTCAACCCCTCAAGCTCGGCTTGTGTGATGGACGACCGTCCGGCCCCCT				495
Query 421	TTGGGGGCGGGACGCGCCGAAAAGCAGTGGCCAGGCCCGGATTCCGGCTTCTGGGGC				480
Sbjct 496	TTGGGGGCGGGACGCGCCGAAAAGCAGTGGCCAGGCCCGGATTCCGGCTTCTGGGGC				555
Query 481	AATGGGCAATCAAAACAGCGCCCTCAGGACCGGCCCTCTGGCCTTCCCCCAAATCTCTC				540
Sbjct 556	AATGGGCAATCAAAACAGCGCCCTCAGGACCGGCCCTCTGGCCTTCCCCCAAATCTCTC				615

**Figure (2): Basic local sequence alignment analysis of local Trichophyton sp. isolate-1 with NCBI-BLAST Trichophyton verrucosum at 100% identity.**

phylogenetic of *trichophyton rubrum* done in the NCBI-BLAST site are Homology sequence identity by use rRNA gene highly sensitivity to accuracy molecular detection see table (5).

**Table (5): NCBI-Blast Homology sequence identity for rRNA gene in Trichophyton sp. isolate-2 with NCBI-BLAST Trichophyton spp.:**

Sequences producing significant alignments:

Select All None Selected 0

	Max score	Total score	Query cover	E value	Ident	Accession
1. <a href="#">Trichophyton rubrum genomic rRNA gene (AJ270806.1)</a>	976	1823	100%	0.0	100%	Query_183681
2. <a href="#">Trichophyton raubitscheki genomic rRNA gene (AF170468.1)</a>	964	1933	100%	0.0	99%	Query_183687
3. <a href="#">Trichophyton lanai genomic rRNA gene (AF170460.1)</a>	964	1861	100%	0.0	99%	Query_183685
4. <a href="#">Trichophyton megninii genomic rRNA gene (AF170464.1)</a>	926	1879	100%	0.0	98%	Query_183686
5. <a href="#">Trichophyton circumvolutum genomic rRNA gene (AJ270791.1)</a>	922	1717	100%	0.0	98%	Query_183684
6. <a href="#">Trichophyton violaceum genomic rRNA gene (AJ270795.1)</a>	910	1687	100%	0.0	97%	Query_183682
7. <a href="#">Trichophyton vernicosum genomic rRNA gene (U614528.1)</a>	686	1358	91%	0.0	92%	Query_183680
8. <a href="#">Trichophyton interdigitate genomic rRNA gene (AJ270790.1)</a>	677	1409	100%	0.0	88%	Query_183683

Trichophyton rubrum genomic rRNA gene (AJ270806.1)

Sequence ID: Query\_183681 Length: 720 Number of Matches: 58

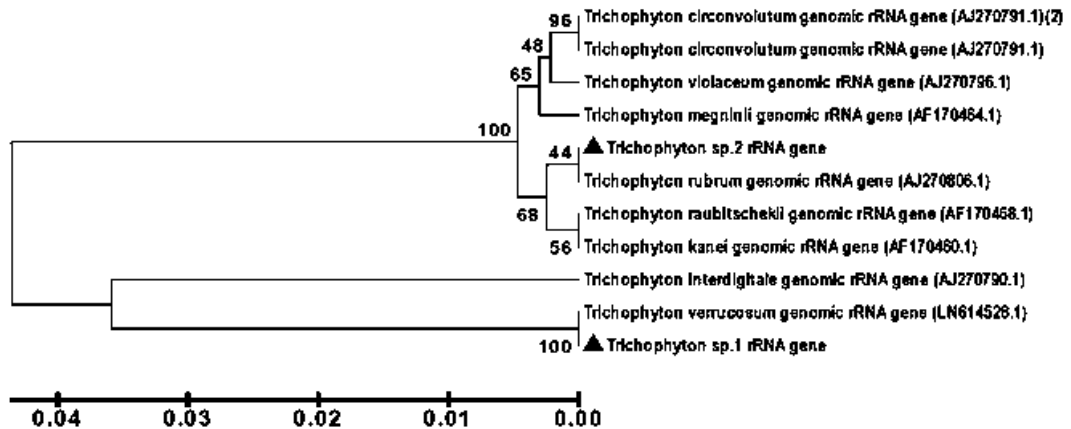
Range 1: 123 to 663 [Graphics](#) ▼ Best Match

Score	Expect	Identities	Gaps	Strand
976 bits(1082)	0.0	541/541(100%)	0/541(0%)	Plus/Plus
Query 1	TCTTETCTACCTCACCCGGTTGCTTCGGCGGGCCGGCTCCCCCTGCCAGGGAGAGCCGT	68		
Sbjct 123	TCTTETCTACCTCACCCGGTTGCTTCGGCGGGCCGGCTCCCCCTGCCAGGGAGAGCCGT	182		
Query 61	CCGGCGGGCCCTTCTGGGAGCCTCGAGCCGGACCGCCCGCCGGAGGACAGACACCAA	120		
Sbjct 183	CCGGCGGGCCCTTCTGGGAGCCTCGAGCCGGACCGCCCGCCGGAGGACAGACACCAA	242		
Query 121	GAAAAAATTCCTGAAGAGCTGTGAGCTGAGCGTTTAGCAAGCACATCAGTTAAAAC	180		
Sbjct 243	GAAAAAATTCCTGAAGAGCTGTGAGCTGAGCGTTTAGCAAGCACATCAGTTAAAAC	302		
Query 181	TTCAACAACGGATCTCTTGGTTCGGCATCGATGAAGAACGACGCAATGCGATAAGTA	240		
Sbjct 303	TTCAACAACGGATCTCTTGGTTCGGCATCGATGAAGAACGACGCAATGCGATAAGTA	362		
Query 241	ATGTGAATTCGAGAATTCGGTGAATATCGAATCTTTGAAAGCACATTGCGCCCTCTGGC	300		
Sbjct 363	ATGTGAATTCGAGAATTCGGTGAATATCGAATCTTTGAAAGCACATTGCGCCCTCTGGC	422		
Query 301	ATTCCGGGGGCATGCTGTTGAGCGTCAATTCACCCCTCAAGCCCGGCTTGTGTGAT	360		
Sbjct 423	ATTCCGGGGGCATGCTGTTGAGCGTCAATTCACCCCTCAAGCCCGGCTTGTGTGAT	482		
Query 361	GGACACCGTCCGGCCCTCCCTTCGGGGCGGGACGGCCCGAAAGCAATGGCCAGGC	420		
Sbjct 483	GGACACCGTCCGGCCCTCCCTTCGGGGCGGGACGGCCCGAAAGCAATGGCCAGGC	542		
Query 421	CGCGATCCGGCTTCTTAGGCGAATGGCGAGCCAAATTCAGCGCCCTCAGGACCGCCGCC	480		
Sbjct 543	CGCGATCCGGCTTCTTAGGCGAATGGCGAGCCAAATTCAGCGCCCTCAGGACCGCCGCC	602		
Query 481	CTGGCCCCAATCTTatatatatatatatatCTTTTCAGSTTGACCCTGGATCAGGTAGG	540		
Sbjct 603	CTGGCCCCAATCTTatatatatatatatatCTTTTCAGSTTGACCCTGGATCAGGTAGG	662		
Query 541	G 541			
Sbjct 663	G 663			

**Figure (4): Basic local sequence alignment analysis of local Trichophyton sp. isolate-2 with NCBI-BLAST *Trichophyton rubrum* at 100% identity.**

### Phylogenetic analysis

Analysis of Phylogenetic tree has done depend on the clone rRNA, a that used for final detection of *trichophyton* spp. draw a tree for trichophyton by Phylogenetic analysis of rRNA gene sequences has become the principal method for knowing prokaryotic phylogeny. Our result show the phylogenetic tree has done according to these isolates see figure(3) and Table(6).



**Figure (3):** Trichophyton spp typing family analysis of Phylogenetic tree was created by using method named (UPG) Unweighted Pair Group with in (MEGA software 6.0 version).

**Table(6):** Show names of strains of isolates that sent to NCBI for sequencing and its codes (Accession number ) of two isolates that selected in this work.

Fungal isolate		Accession number
<i>Trichophyton verrucosum</i>	1- BankIt1987271 Seq1	KY549929
<i>Trichophyton rubrum</i>	2- BankIt1987271 Seq2	KY549930

## Discussion :

The ringworm is a cutaneous disease caused by several genera of fungi including microsporium, epidermophyton, and trichophyton that can live on keratinized tissues in the skin are affected by this invasion (25)(26).

The result of study in the table (2) revealed that Percentage of total isolated trichophyton spp are (34.2%) by use classical media culture, including isolation, staining, and culture ; that was near to results of (27) where was (31%).

In our report too the percentage of trichophyton spp (100%) when it used molecular methods (polymerase chain reaction ) was (100%). while prevalence in (27) was less than our results where it was 17(42.5%)(28).

(29) study Molecular aspects (PCR) of total of trichophyton spp it was (96%) was same to our report results (30).

Percentage of *Trichophyton rubrum* that diagnosed in our study are (33.3%) , that compatibility with (29) that show it was (32.5%) as percentage (29) look at table(3).

Percentage of *Trichophyton verrucosum* that tested in our study is (66.6%) ;That different from (31) that found *T. verrucosum* as percentage (99%) that conserved more than our values(31).

However, Percentage of *Trichophyton verrucosum* was close to our results where was (71.3%) as in (32)(33).

(34)found *T. verrucosum* percentage for the ringworm was (85%) as percentage in calves; its considered more little from ours.

Different percentage, Incidence and prevalence range are contract depend on age of young animals particularly the calves susceptible for the ringworm more than adults ; and this disease may be accurate due to a poor immune system and the high pH of the skin in young animals (35)(36).

The humidity and warm weather of environment and climate could have preferred to the growth and development of fungal spores there by help to replication and infection the fungus in the animals and produce the outbreaks in the population(37)(38).

Finally, The host species; the geographical factors and environmental conditions are main causes to formed contract values phenomones (39)(40).

*T. verrucosum* has been slow growing, white color, cottony consistency, and its yellow pigment. These signs are consistent with the clinical findings of (41) and (42). In our research, so the microscopic examination revealed hyphae thread and small conidia that were adhesion with the hyphae, that same with the study of (31).

Percentage of infection in mature cattle was less than the calves and that like to our results (35) (43), and (44) .

Genomic sequence identification showed that the isolates and nucleotide of *Trichophyton rubrum* show similarity with *Trichophyton raubitschekii* (99%), *Trichophyton kanei* (99%) and *Trichophyton megninii* (98%). while *Trichophyton verrucosum* show likely similarity with *Trichophyton megninii* (93%), *Trichophyton circonvolutum* (92%) and finally likely with *Trichophyton interdital* (93%).

(45) reported for the first time *T. rubrum* var. *raubitschekii* isolated from the toe nail of a hypertensive from old women living in Algeria presenting. The molecular investigation from the isolate led to (99%) of homology with both *T. raubitschekii* JX827168.1 and *T. rubrum* FM178326.1. As previously reported, showed that *T. rubrum* was genetically identical same to *T. rubrum*. (45).

While (49) found the tree of genetic family analysis of *T. rubrum* by use (CHS1) gene that extracted from the isolates detect more than (99%) genome high likely between human and canine isolates of *T. rubrum* then the tests mention that isolates are same sources (46).

Percentage of similarity between our isolates of this study with phylogenic tree analysis database is (100%) by use mega software by use rRNA gene.

(47) found The symmetrical percentage sequencing was more than (97%). according to sources that sequences of new strains, sequencing supply a very sensitive and accurate and he conceded very good method for the knowing of dermatophytes(47).

Using pairwise nucleotide comparisons, a mean match of 81% was noticed among (29) dermatophyte species, with inter-species variety range from 0 to 200 nucleotides(48) ,while our sequencing was same at 100% with the sequence in data base.

This study suggested phylogenetic tree analysis based on rRNA gene, by use primer can be used for confirmative detection of *trichophyton verrucosum* and *trichophyton rubrum* strains and determine the close relationship between These results with informations of data base and showed highlight the importance of detection of trichophyton and it relationship with the cattle that effected with the ringworm disease bu phlogenic analysis tree.

#### Reference :

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# التعرف الجزيئي على بعض مسببات مرض القوباء الحلقية المعزولة من الابقار في مدينة الديوانية

## تقرير الاصلية

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